A Mendelian randomization study on the effect of 25-hydroxyvitamin D levels on periodontitis

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Summary sentence: The current study does not support a protective association between genetically predicted 25-hydroxyvitamin D and the risk of periodontitis.

Running title: 25-hydroxyvitamin D and periodontitis

Abstract

Background: 25-hydroxy vitamin D (25OHD) levels have been proposed to protect against periodontitis based on in vitro and observational studies but evidence from long-term randomized controlled trials (RCTs) is lacking. This study tested whether genetically proxied 25OHD is associated with periodontitis using Mendelian randomization (MR).

Method: Genetic variants strongly associated with 25OHD in a genome-wide association study (GWAS) of 417,580 participants of European ancestry were used as instrumental variables, and linked to GWAS summary data of 17,353 periodontitis cases and 28,210 controls. In addition to the main analysis using an inverse variance weighted (IVW) model, we applied additional robust methods to control for pleiotropy. We also undertook sensitivity analyses excluding single nucleotide polymorphisms (SNPs) used as instruments with potential pleiotropic effects and used a second 25OHD GWAS for replication. We identified 288 SNPs to be genome-wide significant for 25OHD, explaining 7.0% of the variance of 25OHD levels and providing ≥90% power to detect an odds ratio (OR) of ≤0.97.

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Results: MR analysis suggested that a 1 standard deviation increase in natural log-transformed 25OHD was not associated with periodontitis risk (IVW OR = 1.04; 95% confidence interval (CI): 0.97-1.12; *P*-value = 0.297). The robust models, replication, and sensitivity analyses were coherent with the primary analysis.

Conclusions: Collectively, our findings suggest that 25OHD levels are unlikely to have a substantial effect on the risk of periodontitis, but large long-term RCTs are needed to derive definitive evidence on the causal role of 25OHD in periodontitis.

Keywords: "Vitamin D"[Mesh], "25-Hydroxyvitamin D 2"[Mesh], "Periodontitis"[Mesh], "Mendelian Randomization Analysis"[Mesh]

Introduction

Periodontitis is a microbially-associated inflammatory disease of the tooth-supporting tissue that affects approximately 50% of the adult population, with 10% suffering from severe periodontitis [1]. Its features include the loss of periodontal tissue support, manifested through clinical attachment loss and radiographically assessed alveolar bone loss, and the presence of periodontal pocketing and gingival bleeding [2]. Subgingival bacterial dysbiosis, cigarette smoking, and diabetes mellitus are established risk factors for periodontitis [3, 4]. Dietary factors may contribute to the immune-inflammatory response involved in the occurrence of periodontitis [3, 5].

Vitamin D refers to a group of lipid-soluble compounds that can be derived from sunlight exposure or through food intake [6]. Vitamin D is bound to vitamin-binding protein, which is hydroxylated, via 25-hydroxylase, to 25-hydroxy vitamin D (25OHD) (calcifediol) in the liver [6]. 25OHD concentrations are measured routinely to determine the individual vitamin D status. Vitamin D is involved in bone metabolism, has a role in the innate and adaptive immune response, and displays anti-inflammatory and anti-microbial effects [7 8]. Because vitamin D is involved in mineral density and immune response, it is plausible to assume that vitamin D affects periodontal disease. The in vitro and observational literature, in part, suggests a link between vitamin D levels and periodontitis [9, 10]. The *CYP27A1* gene, which encodes 25-hydroxylase, has been found in gingival fibroblasts and periodontal ligament cells [9, 11]. In periodontitis models, 25OHD injection inhibited infection with *Porphyromonas gingivalis*, suggesting that vitamin D deficiency contributes to periodontitis [12, 13].

Most of the available observational studies are cross-sectional or case-control and revealed an association between lower 25OHD levels and poorer periodontal health status [9, 10]. However, these are in contrast to prospective studies, which do not support an association, especially those studies using clinical indicators such as bleeding on probing and clinical attachment loss [10, 14, 15]. The reported observational associations between 25OHD and periodontitis may be causal, but are susceptible to confounding bias, as lower vitamin D levels co-occur with various risk factors of periodontitis, and reverse causality. In recent years, Mendelian randomization (MR) has received increasing attention for addressing some limitations of conventional observational studies to study long-term exposures [16]. Because randomized trials establishing a causal effect of

long-term exposure to 25OHD are not available, MR offers additional evidence complementary to conventional epidemiological studies. We conducted an MR study using summary data obtained from two recently published large meta-analyses of genome-wide association studies (GWAS) of 25OHD and one GWAS of periodontitis [17-19], following guidelines for performing and reporting MR studies [20, 21].

Materials and Methods

MR uses genetic variants that are associated with the exposure of interest but are independent of factors that may confound conventional observational analysis [16, 22]. MR applies an instrumental variable (IV) framework, using the genetic variant as the IV. A valid IV has to satisfy three assumptions [22]. First, the IV is robustly associated with the exposure ("relevance"). Second, the IV does not share common causes with the outcome ("exchangeability"). Third, the IV affects the outcome only through its effect on the exposure ("exclusion restriction"). MR draws on Mendel's laws of segregation and independent assortment, whereby genetic variants are allocated independently of environment and other genetic factors (except those inherited through linkage disequilibrium (LD))[16]. Germline genetic variants are inherited randomly and fixed at conception, and consequently, factors confounding the exposure-outcome relation cannot affect the genetic variant. Genetic variants are also not affected by the outcome, and therefore avoid reverse causation. MR typically uses single-nucleotide polymorphisms (SNP) that are identified and replicated through large-scale GWAS. This study applies Two sample MR analysis to summary SNP-level estimates of the genetic variant-exposure and genetic variant-outcome associations [22].

To assess whether genetically proxied 25OHD levels are associated with odds of periodontitis, we identified the LD-independent (r^2>0.01) SNPs associated with 25OHD from a GWAS in 417,580 Europeans [18] (Table 1, Supplementary Table 1). To satisfy the relevance assumption, we chose SNPs which were associated with 25OHD levels at a level of genome-wide significance (*P*-value < $5x10^{-8}$) [22]. To further verify the relevance assumption, we computed the F statistic and the proportion of the variance of 25OHD explained by all SNPs [23]. The 288 SNPs for 25OHD had a minimum F statistic of 29.8 and explained 7.0% of the phenotypic variability.

In a secondary analysis, we additionally used LD-independent, genome-wide significant SNPs from another 25OHD GWAS in 443,734 Europeans [17] (Supplementary Table 1). We extracted estimates of the effects of the 25OHD-associated variants on periodontitis from a GWAS of 11 European studies, totaling 17,353 cases and 28,210 controls [19]. Periodontitis cases were classified by either the Centers for Disease and Control and Prevention/American Academy of Periodontology [24], the Community Periodontal Index (CPI) [25] case definition, or through study participant reports of diagnosis of periodontitis [19]. Details on the demographics of the cohorts and the measurements can be found in the respective publications [17-19]. Ethical approval was granted for each of the cohorts and informed consent was obtained from all participants prior to participation.

Statistical analyses

A priori statistical power was calculated [26]. Given α=5%, the primary IVW analysis had ≥90% power when the expected ORs for periodontitis was ≤0.97. The main analysis used a multiplicative random

effects inverse-variance weighted (IVW) model to meta-analyze Wald ratios for the effect of a 1 standard deviation (SD) increase in natural log-transformed 25OHD (corresponding to a 29-nmol/l change in 25OHD levels [18]) on periodontitis. Wald ratios were obtained by dividing the per-allele SNP-periodontitis effects by the per-allele SNP-25OHD effects. The presence of associations between genetic variants and potential confounders might violate the exchangeability and/or exclusion restriction assumptions. The plausibility of the two IV assumptions was examined by searching the PhenoScanner database for previously reported associations of our SNPs (and LD-proxies) with potential confounders. As a sensitivity analysis, MR estimates excluding variants associated with potential confounders were calculated. Violations of the exclusion restriction assumption can also occur via horizontal pleiotropy, whereby the IV affects the exposure and outcome but through different pathways. We examined potential horizontal pleiotropy by testing for heterogeneity of the effects of the instruments using the Cochran Q and I_{GX}^2 statistics, applied the MR Egger intercept test of directional pleiotropy, the outlier test using the MR pleiotropy residual sum and outlier (MR-PRESSO) [27], the leave-one-out analysis to assess whether the IVW estimate was driven by a single SNP, and applied various pleiotropy-robust MR methods (penalized weighted median, Radial regression, MR-PRESSO) [27-29]. Analyses were performed using the TwoSampleMR (0.5.6), MRPRESSO (1.0), and MendelianRandomization (0.5.1) packages in R, version 4.0.5. The study was not preregistered.

Results

The results of the MR analyses are shown in Table 2. We did not find evidence supporting an association between genetically proxied 25OHD and risk of periodontitis (IVW OR per 1-SD increment in $\log_e$-25OHD = 1.04; 95% confidence interval (CI): 0.97-1.12; *P*-value = 0.297). The secondary analysis using SNPs from a second 25OHD GWAS did not suggest an association between genetically proxied 25OHD and risk of periodontitis (IVW OR per 1-SD increment in $\log_e$-25OHD = 1.03; 95% CI: 0.89-1.18; *P*-value = 0.708) (Table 2).

In the PhenoScanner search, we found previous reports of associations with serum lipids, blood, anthropometric, body composition, cardiovascular, blood pressure, gastrointestinal, diabetes, renal, inflammatory, bone mineral density, and autoinflammatory traits (Supplementary Table 2). We did sensitivity analysis excluding 49 SNPs, which were associated with either of these traits, and found an estimate similar to the original IVW analysis (OR = 1.07; 95% CI: 0.91-1.16; *P*-value = 0.121). There was no evidence of heterogeneity in the main IVW analysis (Supplementary Table 3). The intercept estimated from the MR Egger regression was centered around zero and provided no support for unbalanced pleiotropy (Supplementary Table 3). Using MR-PRESSO, we found no evidence for pleiotropy (*P*-value global test = 0.891). IVW leave-one-out analyses did not identify any leverage points with high influence. Evaluation of MR estimate under other pleiotropy-robust models showed consistency with the original IVW estimate (Table 2).

Discussion

The results of this MR study do not support a causal association between genetically determined change in 25OHD levels and the risk of periodontitis. The study was sufficiently powered to test small effects and produced consistent estimates using different MR methods and in sensitivity and replication analyses. The majority of the available observational studies applied cross-

sectional or case-control designs and found an inverse association between 25OHD levels and periodontitis [10, 30-40]. Prospective studies, however, did not show associations between 25OHD levels and the occurrence or progression of periodontitis [9, 10]. For example, a population-based cohort of middle-aged and older Germans suggested no association between serum 25OHD levels and changes in clinical attachment loss over 5.9 years [15]. Likewise, the U.S. OsteoPerio Study did not observe associations between 25OHD and 5-year change in clinical attachment loss and probing depth, alveolar bone loss, or tooth loss due to periodontal disease [14, 41]. The result of the present MR analysis is generally in agreement with well-conducted prospective observational studies on the role of 25OHD in periodontitis.

Several potential limitations need to be considered. The periodontitis GWAS [19] used a broadly defined phenotype, including clinical criteria and reported diagnosis, which might have introduced outcome misclassification, which could have attenuated the MR estimate towards the null. Our analysis assumes a linear relationship between the 25OHD levels and the (log odds) of periodontitis. Quantitative estimates may be misleading if the true relationship is non-linear. However, estimates are still reflective of the presence and direction of the population-averaged causal effect [42]. A protective effect may be only presented in certain population subgroups (e.g. postmenopausal women). Unfortunately, we had no access to individual-level participant data to further test effect modification to identify such subgroups using suitable MR methods [43]. In addition, if the effect of the SNP on 25OHD levels changes over the life course, the MR estimate would represent a biased estimate of the lifetime effect [44]. Previous studies have indicated, for example, that the relevance of sunlight exposure and vitamin D in the etiology of multiple sclerosis is limited to early life [45]. In this scenario, an MR study utilizing genetic variants associated with circulating 25OHD after the critical time window would not detect a sizeable association. One way to minimize this potential bias is to average over multiple SNP effects on phenotype (as done here through multiplicative random effects IVW), assuming that time-dependent effects of multiple instruments average across a lifetime [44]. In the present study, the 25OHD and periodontitis SNP effect estimates were obtained from European (ancestry) studies, thus minimizing the possibility of population stratification bias and increasing the plausibility of the two-sample MR assumption that summary associations derived from comparable populations. Nevertheless, caution is warranted before generalizing findings to other populations. We performed sensitivity analyses to assess and minimize heterogeneity and pleiotropy. Sensitivity analysis failed to find evidence for horizontal pleiotropy. Regarding instrument selection, we used a stringent selection threshold (P-value $< 5 \times 10^{-8}$) to reduce the possibility of weak instrument bias [20].

Conclusion

Although biologic mechanisms suggest that vitamin D levels could be involved in the development of periodontal disease, our results identified no effect of genetically proxied elevation in 25OHD levels on peridontitis risk. Our findings suggest that the available cross-sectional observational studies might have been subject to environmental confounding or reverse causation. MR investigations are worthwhile in providing an alternative line of etiological evidence that complements traditional RCT-based causal inference. Long-term evidence from RCTs that follow individuals for many years is needed to derive more definitive evidence on the causal role of 25OHD in periodontitis [9, 46].

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Declarations

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Conflicts of interest/Competing interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Availability of data and material

The summary statistics of the 25OHD GWAS [17, 18] are available at https:// https://cnsgenomics.com/content/data and https://journals.plos.org/plosmedicine/article/file?type=supplementary&id=info:doi/10.1371/journal.pmed.1003536.s002 (access date: 2021/06/29). The periodontitis GWAS [19] summary data are available at https://data.bris.ac.uk/data/dataset/2j2rqgzedxlq02oqbb4vmycnc2 (access date: 2021/03/09).

Author Contributions

Sebastian-Edgar Baumeister, Stefan Lars Reckelkamm, Hansjörg Baurecht, Michael Nolde, Birte Holtfreter and Anke Hannemann conceived the study deisgn and aquired the publicly available GWAS summary statistics. Sebastian-Edgar Baumeister, Stefan Lars Reckelkamm, Hansjörg Baurecht, and Michael Nolde contributed to the statistical analyses and interpretation of the data. Sebastian-Edgar Baumeister, Stefan Lars Reckelkamm, and Anke Hannemann wrote the initial draft. Sebastian-Edgar Baumeister, Stefan Lars Reckelkamm, Hansjörg Baurecht, Michael Nolde, Birte Holtfreter, Thomas Kocher, Benjamin Ehmke and Anke Hannemann revised the manuscript and approvoed the article.

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Table 1 Description of GWAS used for each phenotype

Pheno-type	First author (year)	Sample size	Population	% female
25OHD	Revel (2020)[18]	417,580	100% Euro-pean	54
25OHD	Manousaki (2020)[17]	443,734	100% Euro-pean	55
Periodon-titis	Shungin (2019)[19]	45,563	100% Europe-an	50

Table 2 Mendelian randomization estimates for the association between 25OHD and periodontitis

Analysis	No. of SNPs	Method	OR[a]	(95% CI)	*P*-value
Primary analy-sis using SNPs from Revez (2020) GWAS	288	Inverse variance weighted	1.04	(0.97;1.12)	0.297
	288	Penalized weighted median	1.06	(0.91;1.24)	0.420
	288	IVW radial	1.04	(0.97;1.12)	0.297
	288	MR PRESSO	1.04	(0.97;1.12)	0.298
Secondary analysis using SNPs from Manousaki (2020) GWAS	65	Inverse variance weighted	1.03	(0.9;1.18)	0.708
	65	Penalized weighted median	1.09	(0.91;1.31)	0.347
	65	IVW radial	1.03	(0.90;1.18)	0.708
	65	MR PRESSO	1.03	(0.90;1.18)	0.709

MR PRESSO, MR Pleiotropy RESidual Sum and Outlier. [a] OR (odds ratio) per one standard deviation increment in log-transformed 25OHD